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ACTION POTENTIAL PROPAGATION AND THRESHOLD PARAMETERS IN INHOMOGENEOUS REGIONS OF SQUID AXONS

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SUMMARY

1. The squid giant axon was used as a model system in which to determine the independent contributions of membrane excitability and diameter changes to threshold parameters and propagation of action potentials in inhomogeneous regions. The membrane excitability of a segment of an axon was altered by changes in the bathing solution, while its effective electrical diameter was increased by the insertion of a low-resistance axial wire. In computer simulations of these experiments, similar alterations were made in the membrane's conductance and axon's diameter.

2. The inflexions in the shapes of action potentials propagating into a region with abrupt decreases in axial resistance become more pronounced when the interval between impulses was shortened. At short intervals, propagation of the second impulse failed. In contrast, reduction of membrane excitability produced inflexion-free changes in action potential shape and allowed a close-following second impulse to pass through the inhomogeneity.

3. A combined decrease in membrane excitability and increase in diameter of the same region exaggerated the changes in action potential shape characteristic of the diameter increase alone.

4. Threshold parameters were obtained from 'stength–duration' excitability relationships measured by injection of current at different points along the axon. When only the membrane excitability was reduced, threshold characteristics changed smoothly from one region of the nerve to another. In contrast, lowering the internal resistance or increasing the diameter in one region of a nerve lowered the time constant of excitation and the threshold for brief (relative to rheobasic) current stimuli in the small-diameter region near the transition while raising them in the larger-diameter region.

INTRODUCTION

Characterization of the electrical properties of nerve cells as the fundamental units of the nervous system has been a major interest of physiologists ever since His and Forel in the later nineteenth century first introduced the 'neurone theory' which was

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later firmly established by the work of Ramón y Cajal (1909). Since that time, many investigations have developed phenomenological descriptions of impulse initiation and propagation in a vast number of different cells. However, we still await a complete description of how the geometry and membrane characteristics of nerve cells affect their electrical function.

Differences in electrical properties between axons and somata have been measured in both vertebrate (Richter, Schlue, Mauritz & Nacimiento, 1974) and invertebrate (Wald, 1972; Horn, 1977) neurones. Some of the effects of geometry on impulse propagation have been investigated in models by Dodge & Cooley (1971), Goldstein & Rall (1974), Khodorov (1974), Ramón, Joyner & Moore (1975), and Dodge (1979), and in experiments by Ramón, Moore, Joyner & Westerfield (1976) and Westerfield, Moore, Ramón & Joyner (1978). Regional differences in the membrane and geometrical properties of axons have been seen in invertebrates (Spira, Yarom & Parnas, 1967) and vertebrates (Waxman, 1971). The results of all these studies suggest that *both* membrane and geometrical inhomogeneities play important roles in determining normal neuronal function. However, the relative and indepedent contributions of such changes in diameter and channel density to impulse excitation and propagation have not been completely determined.

To understand better the roles of a cell's shape and membrane excitability in controlling its function, we present here observations of changes in electrical activity produced by controlled changes in specific cellular properties. We have investigated the independent contributions of membrane properties and cellular structure to impulse initiation and propagation in the squid giant axon. Alterations in the membrane excitation characteristics in selected regions were achieved by treatments with drugs, toxins or low-Na^+ sea water. Local alterations in the effective (or 'electrical') diameter were made by lowering the internal resistance with an axial wire (Cole & Moore, 1960; Ramón *et al.* 1975, 1976; Westerfield *et al.* 1976). This paper describes our studies and observations on action potential thresholds and propagation near such inhomogeneities.

METHODS

Giant axons of *Loligo pealei*, collected at the Marine Biological Laboratory in Woods Hole, Massachusetts, were excised, cleaned and immediately placed in a lucite chamber through which filtered sea water continuously flowed. The temperature of the sea water, monitored with an immersible thermistor (less than 0·5 cm from the axons), was controlled by a Peltier thermoelectric device (between 0 °C and 30 °C to within ± 1·0 °C).

Membrane inhomogeneities

Changes in membrane characteristics were achieved by regional changes in the composition of the bathing medium. The axon chamber was divided into two compartments for different bathing media by a thin rubber membrane made from a prophylactic. Each compartment had its own inlet and drain for the continuously flowing bathing solutions. Tests with water-soluble dyes indicated that there was very little mixing between the two compartments when the bathing solutions were maintained at equal heights. Axons were carefully drawn through a 500 μm diameter hole in the rubber with a fine silk thread, until the length of axon in each compartment was equal. Propagation of action potentials (with normal sea water in both compartments) was not affected by the presence of this membrane.

Some of the experiments required the substitution of artificial sea water (ASW) of various

compositions for the normal filtered sea water. The ASW was buffered with tris(hydroxymethyl) aminomethane (Tris) adjusted to pH 7·3 at 20 °C and to 950 ± 20 mosmol/l. The composition of the ASW was 450 mM-NaCl, 10 mM-KCl, 50 mM-$CaCl_2$, 10 mM-Tris. To make the La^{3+} and tetrodotoxin solutions, $LaCl_3$ (2–10 mM) or tetrodotoxin (10^{-7} to 10^{-8} M) were added to the ASW. Low-Na^+ media were made by reduction of NaCl from 450 to 225 mM and replacing that removed with either 315 mM-sucrose or 225 mM-Tris-Cl.

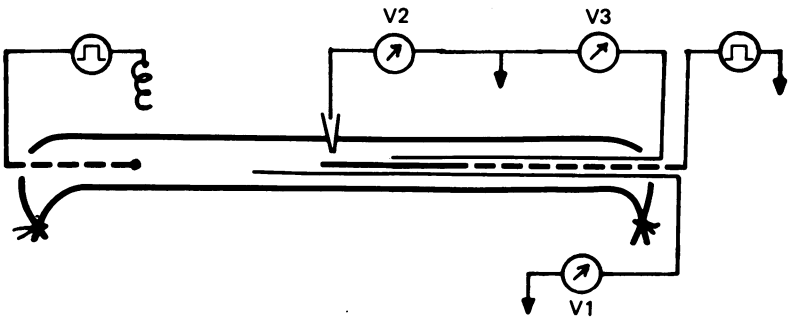

Fig. 1. The experimental arrangement, showing the axon and the electrodes. A low-resistance axial wire (thick unbroken line in the axon) was used to shunt the axoplasmic resistivity and to inject current. Transmembrane potential was measured with 'piggyback' capillaries or with glass micropipettes. An independent axial probe was inserted from the opposite end and was used either for stimulation or for measurement of transmembrane potential.

Electrical equivalent of geometric inhomogeneities

The insertion of an axial wire into an axon lowers the axial resistance by short-circuiting the axoplasm. Careful plating of the wire surface with platinum black can give it a low enough resistance to cause the surrounding axon to have a reduced space constant, characteristic of an increase in diameter. Except for a short transitional region (Cole & Moore, 1960; Fig. 8) the membrane surrounding the wire is nearly isopotential; activity along the axon surrounding the wire is synchronous (del Castillo & Moore, 1959).

We platinized 75 μm or 125 μm (diameter) platinum wires over a length of 10–40 mm to give a low surface resistance (2–4 Ω cm^2). These wires were also used for stimulation with current pulses and for measurement of intracellular potential. 'Piggyback' capillaries (Wang, Narahashi & Scuka, 1972) or glass micropipettes were also used to measure transmembrane potentials. The placement of the electrodes is illustrated schematically in Fig. 1.

Simulations

Computer simulations of the experiments used the Hodgkin–Huxley (1952) equations for membrane conductance and the partial differential cable equations and integration method (Crank & Nicolson, 1947) previously described (Moore, Ramón & Joyner, 1975). The boundary conditions were specified to simulate the ends of the axon or the transition region from one diameter to another (Joyner, Westerfield, Moore & Stockbridge, 1978).

For each simulation, the effective diameter change which was electrically equivalent to the resistance change produced by the axial wire was calculated from the measured characteristics of the axon and the wire. The model had 120 isopotential segments divided equally between the two axonal regions, which extended more than five resting length constants in both directions from the diameter change.

The following constants are used: x (μm), longitudinal distance; λ (μm), the electrical length constant at resting potential; τ_e (ms), the time constant of excitation derived from the strength–duration relationship (Guttman, 1966); τ_m (ms), the membrane time constant derived from the specific membrane resistance and capacitance; τ_0 (ms), the time constant of the potential change in response to current injected at a point in a completely uniform axon; τ_1 (msec), the time constant (measured like τ_0) in non-uniform axons.

RESULTS

Propagation through inhomogeneities

Effect of lower internal resistance. Insertion of an axial wire with low surface resistance locally reduces the axial resistance (the electrical change associated with a diameter increase) with an abrupt change in the cable properties of the axon (Cole & Moore, 1960). When action potentials propagate into the transition region from high to lower axial resistance, they exhibit a dramatic change in their shape. Inflexion points appear (e.g. Goldstein & Rall, 1974; Ramón *et al.* 1976) as can be seen in the lower traces of Fig. 4.

Action potential parameters were measured thoughout the transtion from high to low internal resistance. As an impulse in the normal axon entered the transition region, its maximum rate of rise became smaller ($23\% \pm 5\%$; $n = 5$) while its duration at half-maximum amplitude was longer ($14\% \pm 2\%$). Its peak amplitude became smaller but there was a great deal of variation in this particular parameter from preparation to preparation.

Effect of reduced membrane excitability: shifting rate constants and decreasing conductances. The trivalent cation La^{3+} has been shown to reduce the leakage and shift the voltage dependence of the membrane conductances in a manner which decreases excitability and raises threshold by reducing the maximum conductance of both the Na^+ and K^+ channels (Takata, Pickard, Lettvin & Moore, 1966; Jaslove, 1980).

The shape of impulses changed when they propagated into a region of axon bathed in La^{3+} sea water. Fig. 2 shows simultaneous records of an action potential propagating from a segment of axon in normal sea water (lower trace) into a region bathed by a La^{3+} (10 mM) solution (upper trace). These records, obtained very near the transition between the solutions, show that the shape of the action potential changes smoothly (without the inflexions seen near the axial resistance changes) as it propagates into a region with different membrane conductance parameters.

Because there was a marked difference in spike shape on either side of the rubber partition, Fig. 2 also gives assurance of very little mixing between the two pools of the chamber. Furthermore, impulses recorded in the normal sea water near the inhomogeneity (lower record) showed little or no change when La^{3+} was introduced into the other bathing pool.

Effect of reduced membrane excitability: reduction of Na^+ currents. Membrane excitability was reduced also by lowering the concentration of Na^+ bathing the nerve. This reduced the peak Na^+ current by shifting the Na^+ equilibrium potential (Hodgkin & Katz, 1949; Moore & Adelman, 1961). The shape of an impulse changed as it propagated from ASW into a region with low external Na^+; the maximum amplitude and the maximum rate of rise decreased while there was little or no change in duration. Reduction of the Na^+ concentration to one-half (substituting either sucrose or Tris) decreased the maximum amplitude by $18\% \pm 2\%$ (average of five experiments) while the maximum rate of rise decreased $46\% \pm 3\%$. These values compare favourably with those obtained by Hodgkin & Katz (1949) of 23 % and 47 %, respectively. Action potentials which propagated through the transition region near

the partition showed only smooth shape changes, never any signs of inflexion points or propagation failures.

The peak Na^+ conductance was reduced also by addition of very small amounts of tetrodotoxin (Moore, Blaustein, Anderson & Narahashi, 1967) to one half of the axon. Changes like those in low Na^+ were seen but the results were not as reproducible as those obtained with lowered Na^+.

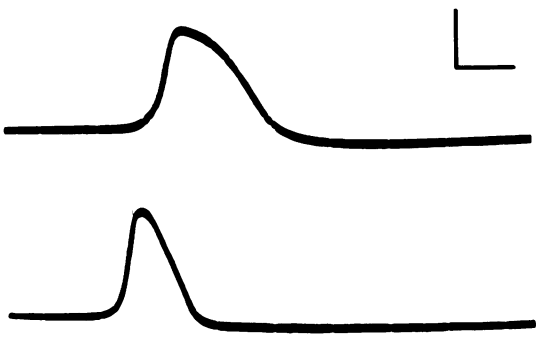

Fig. 2. The effect of abrupt reduction in membrane excitability on an action potential propagating through the transition from ASW (lower trace) into a region containing La^{3+} (10 mM) (upper trace). Axon diameter, 380 μm; resting potential, -62 mV; temperature, 10 °C. Scale calibrations are 50 mV and 1 ms.

Computer simulations of these experiments gave similar results. The effects of La^{3+} were simulated by shifting the voltage dependence of the Hodgkin & Huxley (1952) rate constants, and the Na^+ conductance of the model was reduced by lowering the value of $\bar{g}_{Na}$. When these alterations in the membrane model were assigned to part of the model axon, action potentials propagated smoothly through the transition without inflexions.

Combined effects of membrane and axial resistance inhomogeneities. The characteristic changes in impulses seen upon reduction of excitability were quite different from those associated with a reduction in axial resistance, and raised the question as to whether such changes might be additive, producing dramatic changes in shape or propagation failure. The question was easily answered by altering the conductance of the membrane surrounding the wire. The region of the axon containing an axial wire of moderate resistance (50 Ω cm^2) was bathed in a solution of La^{3+} (10 mM) or half-normal Na^+. The tip of the axial wire extended less than 200 μm (0·03 $\mu\lambda$) into the compartment containing the normal ASW.

Fig. 3*A* shows that the axial wire with moderate resistance produced little or no change in the shape of the spike as it propagated from the normal axon (lower record) into that containing the wire (upper record). However, replacement of the ASW in the compartment surrounding the wire with La^{3+} sea water caused the spike duration to increase and the characteristic bump to appear on the rising phase of the action potential (upper record of Fig. 3*B*). At the same time the inflexion point appeared in the falling phase of the impulse approaching the transition from the normal region (lower record of Fig. 3*B*).

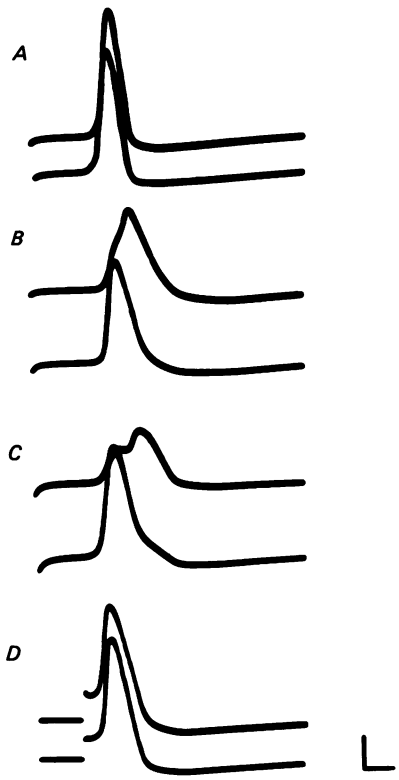

Fig. 3. Impulse propagation into a region of moderately low internal resistance whose membrane was treated to reduce its excitability. The lower trace in each frame was recorded in the region of normal axon and the upper trace was recorded in the region containing the axial wire with a moderately high surface resistance (50 Ω cm²). Frame *A* was recorded with ASW surrounding the axon region with the wire. The records in *B* were taken 2 min and those in *C* 10 min after 10 mM-La^{3+} was applied to the outside of the axon in this region. The region bathed with La^{3+} could be stimulated locally with the axial wire to elicit an action potential as shown in frame *D*. Axon diameter, 400 μm; resting potential, −60 mV; temperature, 12 °C. Scale calibrations are 20 mV and 2 ms.

As the La^{3+} superfusion continued, both inflexion points became more pronounced and, after 10 min, the spike was nearly blocked (upper record of Fig. 3*C*). La^{3+} caused propagation failure without total loss of excitability because good-sized action potentials could be generated by direct stimulation of the region of axon bathed by the La^{3+} with a pulse of current applied through the axial wire (Fig. 3*D*). Similar additive effects were observed when the region of axon containing the wire was bathed in normal sea water with a minute amount of tetrodotoxin (failure at 100 nM) or in a lower-than-normal Na^+ solution. However, in these cases there was a greater loss of excitability measured by direct stimulation as above.

Interstimulus intervals

Effect of lower internal resistance. When an impulse struggles through a region of low safety factor such as a step increase in diameter, the membrane is left temporarily in a condition of reduced excitability. If a second impulse travelling in the small axon

arrives at the junction too soon afterwards, it may fail to propagate into the region of lower resistance, as illustrated in Fig. 4. In each frame the upper trace is the membrane potential at a point 540 μm before the impulse encounters the axial wire, while the lower trace is the transmembrane potential in the membrane surrounding the wire.

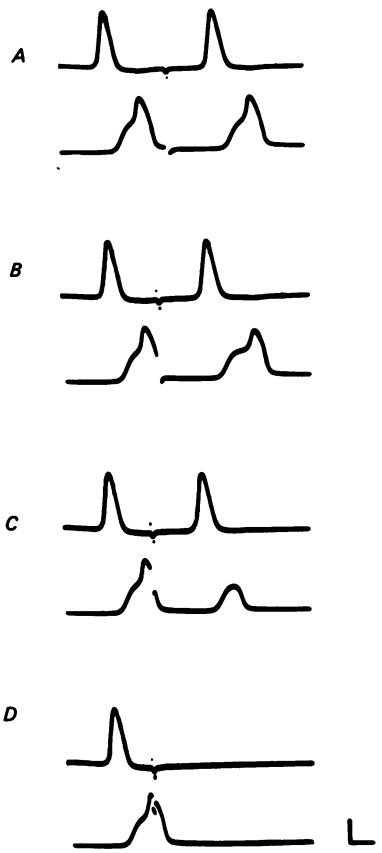

Fig. 4. The effect of interspike interval on propagation of action potentials into the transition region from normal to low internal resistance. The upper trace in each frame was recorded in the region of normal axon between the stimulating electrode and the axial wire. The lower trace in each frame was recorded in the region containing the wire. Paired electrical stimuli were delivered to the region of normal axon with an interstimulus interval of 4·5 ms in *A*, 4·2 ms in *B*, 4·0 ms in *C*, and 3·5 ms in *D*. Axon diameter, 560 μm; resting potential, −65 mV; temperature, 16 °C. Calibration scales are 50 mV and 1 ms.

As the interval of time between a pair of stimuli was decreased from 4·5 ms (Fig. 4*A*), the second spike recorded in the region surrounding the wire decreased in amplitude (4·2 ms: Fig. 4*B*) and finally disappeared (4·0 ms: Fig. 4*C*), leaving only the local response. Further reduction of the interval to 3·5 ms (Fig. 4*D*) led to failure of the second spike even in the region of normal axon (upper trace) and, of course, to the absence of a local response to the second stimulus in the lower trace.

Effect of lower membrane excitability. In similar experiments using stimulus pairs to examine the propagation of the second impulse into regions of reduced membrane excitability, there was no significant change in the shape of the action potential at shorter durations either with La^{3+} or low-Na^+ solution. As the interstimulus interval was reduced, initiation of the second impulse failed at the point of stimulation in the normal region (not in the transition region of reduced excitability).

Input resistance

Effect of lower internal resistance. The relative input resistances of various regions of the inhomogeneous axons were measured by injecting current through the movable internal probe electrode shown in Fig. 1 and by observing the voltage response of the same region of the nerve with a micro-electrode. The voltage change in response to a given current injection was significantly decreased by the presence of an axial wire because of the effective increase in diameter and, thus, effective membrane area.

Effect of lower excitability. Shifting the voltage dependence of the membrane conductances by external application of La^{3+} changed the current–voltage relationship and decreased the resting membrane leakage (Takata *et al.* 1966). The maximum voltage response to hyperpolarizing current pulses was greater after application of La^{3+}; this increase in input resistance was reversible with prolonged rinsing. La^{3+} at 2 mM produced an average increase of $24\% \pm 2\%$ ($n = 8$) in input resistance.

On the other hand, lowering the concentration of Na^+ in the external solution produced little or no change in the current–voltage relationship, consistent with the expectation that reduction of the Na^+ equilibrium potential should not significantly affect the resting input resistance.

Voltage threshold

Effect of lower internal resistance. In a uniform membrane patch the threshold voltage (separating regions of regenerative from passive response) is determined by the density and voltage sensitivity of the ensemble of ionic channels. For squid axon membrane this threshold lies between 15 and 20 mV of depolarization from rest (Guttman, 1966). This is a small fraction of the 25–35 mV depolarization at the inflexion point seen, both in experiments and in simulations, on the rising phase of an action potential propagating into a region of reduced axial resistance. Similar values of maximum depolarization are observed when an impulse fails to invade because the axial wire is too long or is hyperpolarized or because it follows another impulse too closely (as in Fig. 4). Both in our experiments and our simulations we have seen inflexions in the action potential only in the vicinity of a change in electrical load, and never in the presence of a change in excitability. Thus the inflexion point or peak amplitude of an action potential component is not a measure of threshold in a propagating action potential.

Effect of reduction of membrane excitability. In experiments where membrane excitability was decreased in part of the axon by reduction of Na^+ currents (low-Na^+ solutions) or by shifting its voltage dependence (La^{3+} solutions), threshold voltage increased smoothly from the ASW to a higher level on the La^{3+} or low-Na^+ side of the partition. On the average, threshold increased $31\% \pm 6\%$ ($n = 11$) when half the

Na^+ was removed from the external bathing solution, or $22\% \pm 3\%$ ($n = 7$) after addition of 2 mM-La^{3+}.

Current threshold

The current threshold was assayed by finding the minimum current strength required to elicit an action potential at any particular stimulus duration ranging from 10 μs to 100 ms. In order to determine the effect of an abrupt change in internal resistance or membrane excitability on the strength–duration relationship, the stimulating electrode was moved along the axon through the transition at the tip of the wire or the rubber partition.

Effect of lower internal resistance. As the locus of current injection was moved from the normal axon into the region of the low-resistance axial wire, the current threshold increased. Fig. 5 shows the strength–duration curves obtained in the normal axonal region (–7·2 mm and -300 μm from the end of the 12 mm × 125 μm wire) and in the region containing the wire (8·4 mm from its tip). The rheobase current level (the minimum stimulus strength required at long stimulus duration) steadily increased as the electrode moved from the normal axon (bottom curve) into the region containing the axial wire (top). The rheobase in the latter, 8·4 mm from the end of the wire, was about 4 times larger than the rheobase level of the normal axon, while the input resistance (measured as the slope of the current–voltage curve) was about one-third that in normal axon.

The time constant of excitation, τ_e(ms), is given by the intersection of the constant charge (Q) and rheobase (I_0) lines of the strength–duration curve (Guttman, 1966; Jack, Noble & Tsien, 1975). Fig. 5 shows that as the current-injecting electrode moved towards the tip of the axial wire τ_e was reduced (40%; continuous curve, -300 μm) but increased back to normal when the electrode was well within the region containing the wire (upper broken curve, +8·4 mm). The reduction in τ_e near the transition of axial resistance varied considerably in other experiments (averaging about 25%); some of this variability may have been due to differences in the condition of the axons because occasionally there were changes of up to 10% along apparently normal, uniform axons.

The characteristic shape of the strength–duration curve was not affected by the axial wire even though the magnitude and position of the curve were. That is, after normalization to a common rheobase and τ_e the data were well fitted by a single strength–duration curve.

Simulations of the strength–duration experiments were carried out by adding stimulating current pulses of specified strengths and durations to the membrane current of chosen segments. The computer-generated strength–duration curves showed the same characteristic shift of τ_e to smaller values in the normal axon near the transition in diameter. Values of τ_e in different axonal regions are shown in Fig. 6. The abscissa, x (μm), is the distance from the point of the diameter change, normalized to the resting electrical length constant, λ (μm), of the particular axonal region. The actual length of the wire region was approximately 1·5 cm and the normal axonal region was much longer. The geometrical model used in the simulation is represented by the broken line at the bottom of Fig. 6. The continuous curve is the

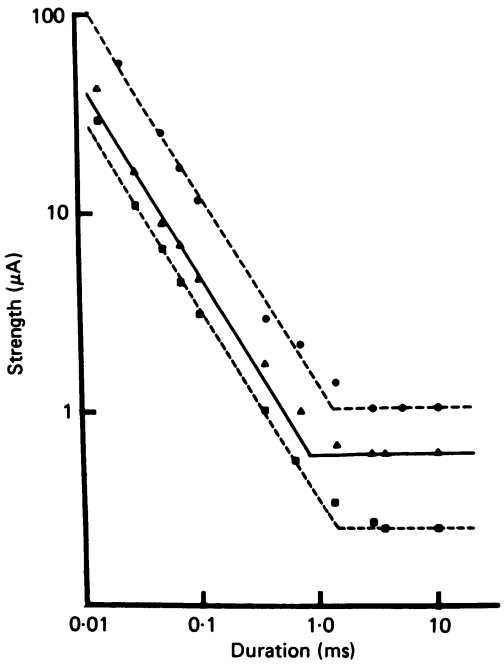

Fig. 5. Strength–duration curves measured in an electrically inhomogeneous axon. The squares are the threshold stimulus strengths which were measured 7·2 mm from the tip of the axial wire in the region of the normal axon. The triangles were measured 300 μm from the tip of the wire. The circles were measured in the region of the wire, 8·4 mm from its tip. Axon diameter, 580 μm; resting potential, −63 mV; temperature, 14 °C.

τ_e predicted by the computer simulation and the points are the experimental values. In the normal axon near the transition, τ_e is faster than anywhere else in the cell. Just beyond the transition in the larger-diameter region τ_e is slowest.

Effect of membrane conductance changes. When the Na^+ concentration was reduced to one-half over the whole length of axon, the rheobase level increased (on the average, 63 % ± 12 %; $n = 4$) but τ_e remained unchanged. In other experiments, where the low-Na^+ solution flowed in only half of the chamber, the rheobase changed smoothly through the transition but no significant shifts in τ_e were observed.

La^{3+} (2 mM) applied uniformly over the entire length of the axon reduced the rheobase current level by 20 % ± 3 % and increased τ_e by 38 % ± 12 % ($n = 7$). When La^{3+} was applied in concentrations up to 10 mM to only one half of the nerve, smooth changes in the rheobase level and τ_e were observed through the transition. This is in marked contrast to the remarkable biphasic change in threshold observed near a change in diameter or internal resistance.

Transient responses

Effect of lower internal resistance. Non-uniformities in internal resistance affected the transient voltage response of the nerve to injection of current at a point.

Fig. 7 shows computer simulations which illustrate changes in the transient

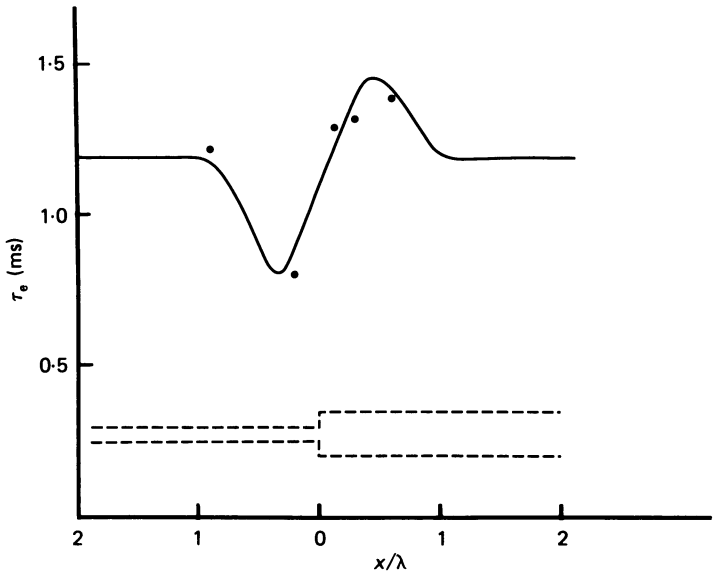

Fig. 6. Effect of 3-fold change in diameter on the time constant of excitation, τ_e. This is plotted as a function of position relative to the transition point, normalized to the resting electrical length constant, λ, which has different values in the two regions. Experimental observations are plotted as points and the curve is drawn from computer simulation of strength–duration experiments in an inhomogeneous axon represented by the dashed lines. Axon diameter, 520 μm; resting potential, −60 mV; temperature, 12 °C; effective diameter increase of axial wire, 3-fold.

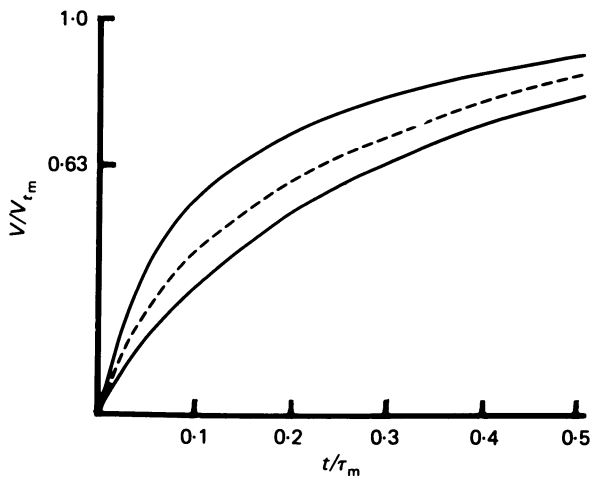

Fig. 7. Simulations of the membrane potential response to a hyperpolarizing current injected near a 3-fold diameter transition, compared with that of a uniform cable (dashed line). The response is faster in the transition (0·23 x/λ) from small to large diameter (upper curve) and slower near a transition (0·23 x/λ) from large to small diameter (lower curve). The steady-state potential responses to current steps are normalized for comparison and the time (t) is normalized to the resting membrane time constant τ_m.

response of a cable with an active (Hodgkin–Huxley) membrane to a hyperpolarizing pulse of current near an abrupt 3-fold change in diameter. The middle (dashed) curve is the voltage response to current injected at a point in a uniform cable. The upper continuous curve is the response to current injected into a segment in the smaller-diameter region of the model at a distance 0·23 x/λ from the transition point. The lower curve is the response of the electrically equivalent segment of the larger-diameter region. The abscissa was plotted in units of time normalized to the resting membrane time constant, τ_m (ms); the steady-state potentials were also normalized to allow direct comparison. The time course of the responses was complex but could be expressed as a sum of exponential terms. The essential point is that the shape of the transient potential response curve is strongly altered by neighbouring changes in diameter, being much faster on one side of the transition and much slower on the other.

Experimentally, the presence of a wire with low surface resistance altered the transient response to injection of current in the transition region of the nerve as predicted by the simulations. When pulses of hyperpolarizing current were injected into the axon at a point, the potential change could be described as a sum of several time constants. For excitation, the fastest or initial time constant, τ_1, dominates and is used in the characterization to be given here.

Near the tip of the wire the normal axon responded more quickly to injected currents than any other region, and the slowest response was observed near the end of the region containing the wire. Fig. 8 shows the initial time constant, τ_1 (ms), (filled circles) of the rising phase of the potential response as a function of distance from the end of the wire, equivalent to a 3-fold increase in diameter. The continuous curve gives the time constants found by a computer simulation of a nerve with uniform membrane properties but with the shape shown by the schematic line-drawing at the bottom of the Figure. For convenience in representation, the time constants were normalized to the time constant of the normal axon region, τ_0 (ms), and the distances were normalized to the resting electrical length constant, λ. The fast time constant of the transient response was affected as far as one (resting) electrical length constant away from the change in diameter. In the normal axonal region, at approximately half a length constant (0·25 cm) from the transition, the membrane potential initially rose twice as fast as in a homogeneous axon. Half a length constant into the lower-resistance or larger-diameter region (0·5 cm) the potential began to rise only 75 % as fast as in a normal axon. At distances one to two length constants from the discontinuity, the transient potential response had nearly returned to normal.

Effect of membrane conductance changes. Reduction of Na^+ to half its normal concentration left the time constants of the membrane potential response unchanged. This invariability was observed in two axons which were uniformly bathed in low-Na^+ solution and in four axons in which the Na^+ concentration was reduced on only one side of the partition. In no case was there any significant change in the time constants of any region of the nerve.

On the other hand, the addition of 2 mM-La^{3+} to the sea water increased the membrane's initial time constant by 41 % ± 5 % ($n = 6$). When La^{3+} was added to only half of the axon, the time constants of the potential response increased smoothly from the normal to treated axon region. The transient response of any part of the

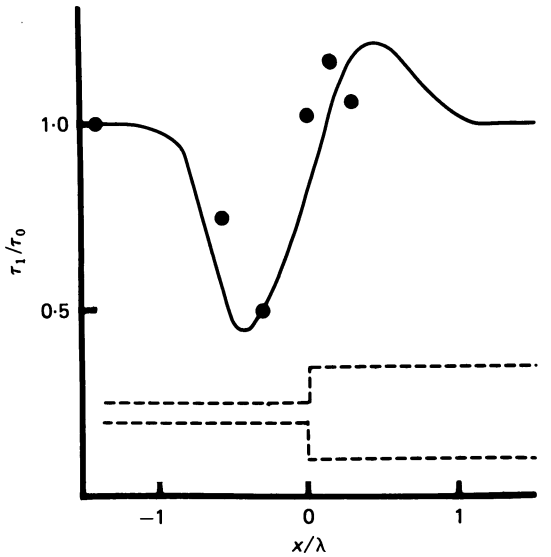

Fig. 8. The effect of an electrical inhomogeneity on the transient response of various regions of a nerve. The time constant, τ_1, of the potential change produced by a hyperpolarizing current is plotted as a function of distance from the 3-fold increase in diameter. The points are the values measured in one experiment and the continuous curve is predicted by the simulation of this experiment. The time constants were normalized to the time constant of the normal axon, τ_0, and the distances were normalized to the resting electrical length constant, λ, which has different values in the two regions. The computer model sketched at the bottom of the Figure extended to $x/\lambda = \pm 5$. Axon diameter, 450 μm; resting potential, −65 mV; temperature, 4 °C; effective diameter increase of axial wire, 3-fold.

nerve was never speeded up by La^{3+}. These observations are consistent with the increase in the membrane resistance on addition of La^{3+} to the medium.

DISCUSSION

The inflexion level is not a measure of threshold

Our results suggest that voltage inflexions of action potentials propagating through inhomogeneous regions are produced in the following way. The larger capacitance of the region of larger diameter requires more current for a longer time (than for the normal diameter) to be charged to threshold for a regenerative response. Joyner, Westerfield & Moore (1980) have measured such currents and charges and shown a 50% increase in Na^+ movement for a 4-fold increase in diameter. Furthermore, to achieve the increased delivery of charge (to the lower-resistance and larger-capacitance load), the action potential is decreased in amplitude and increased in duration (Goldstein & Rall, 1974; Joyner *et al.* 1980). The inflexion in the rising phase of the impulse seen in the transition to the larger-diameter region reflects the difficulty of charging the larger capacitance by the restricted current source of the adjacent segment of normal-diameter axon (with a smaller area and larger axial resistance per unit length). Of course, this is also true in the axial wire experiments because shunting

the axial resistance brings a large membrane area into near isopotentiality and thus increases the capacitance load. The resultant delay in charging is illustrated by the increase in τ_e (Fig. 6) and τ_1 (Fig. 8) in the larger-diameter region of the model and the lower-resistance region of the axon. Thus, the voltage at which the inflexion occurs is related to the electrical load characteristics of the transition region and is *not* a direct measurement of the excitation threshold of the local membrane.

The phenomenon of 'failure of invasion' of the second of two closely spaced impulses can also be understood in similar terms. The refractory process further reduces the 'safety factor' (Jack *et al.* 1975) for propagation of the second impulse into a region of transition from small to large diameter. The relative refractory period of the axon is largely determined by the time for the Na^+ conductance inactivation and the increased K^+ conductance to recover to near normal. It sets the upper limit of the frequency of spikes which can be initiated and propagated by stimuli of a given size above the normal threshold for a single impulse. When the interval between a pair of spikes is reduced (as in Fig. 4) the delay in firing the first impulse at the transition to a larger diameter and the increased duration of the impulse extend the period of reduced excitability. This requires that the next impulse arriving at the transition furnish still more current to ensure propagation through the transition.

In contrast, regional reductions in membrane excitability do not increase the electrical load and action potentials propagate through such transitions without inflexions, showing only gradual and smooth changes in amplitude, rate of rise and the velocity of propagation. Furthermore, these reductions in excitability do not reduce the frequency of impulse propagation as does the increased electrical load.

When either a reduction in membrane excitability or hyperpolarizing current (Ramón *et al.* 1976) is superimposed on a diameter increase, the independent contributions are additive. Not only does the region of increased diameter require more time and charge to depolarize by a given value, but also the threshold voltage is raised. Our observations show that the reduction of excitability by addition of La^{3+} (Fig. 3) exaggerates the effect of a lower internal resistance. These records are similar to those in which a resistance decrease was accompanied by hyperpolarization. Furthermore, both of these combinations are similar to the effects of a still greater increase in diameter alone (Ramón *et al.* 1975; Westerfield, Joyner & Moore, 1978).

Excitation time constants

The time constant of excitation, τ_e, is related to the time constants of the membrane potential change produced by injected current. A region of nerve whose membrane potential rises quickly upon injection of a current step will reach threshold sooner. When a cell is stimulated with a point-source of current, some of the applied charge is shunted to regions at a distance from the point of current injection (Rall, 1969; Jack *et al.* 1975). In a normal region near an increased diameter, the voltage rises more rapidly to its steady-state value because the resistance shunting the membrane capacitance is smaller and, thus, the time constant in this region is shorter (e.g. Fig. 7). The converse is true in the larger-diameter region near the transition: the speed of the voltage response is slowed.

In contrast, when resting membrane resistance is increased without a capacitance change, the transient response (e.g. with La^{3+}) changes smoothly from the normal to treated region of the membrane.

Although τ_e is an important threshold parameter, it is only one of several factors that determine where and when a spike is initiated in a cell. Thus, the experiments presented here cannot address the issue of spike initiation. What they have shown are the relative contributions of inhomogeneous membrane and cable properties to excitation parameters. The essential point is that time constants and threshold parameters change smoothly through transitions in which the membrane properties are different, but undergo unique perturbations near changes in axial resistance or diameter. The fact that τ_e and τ_1 are minimal in an axon near an increase in diameter means that at this location a brief, strong pulse is relatively more effective than a long, rheobasic stimulus. This may have implications for the initiation of impulses in initial segments of axons with fast excitatory synaptic potentials and slow inhibitory potentials.

Our results support the view that regional inhomogeneity in the cable properties of a neurone can produce different apparent thresholds. Furthermore, we have shown that the geometry of the initial segment/somatic transition region of many neurones is sufficient to produce differences in the apparent threshold (voltage level of inflexion) of these two regions even when the membrane properties are uniform. Such regional differences in apparent threshold need not indicate differences in membrane properties. This is because the level of inflexion in a propagating action potential is not an accurate representation of threshold in the soma, but is caused by non-uniform cable properties.

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